

accordance with the Examiner's recommendations, and the Applicant's response to the rejections are provided herein.

**(1) Amendment to the Specification (37 CFR 1.121(b))**

There is an error on page 29 of the specification. In the sentence beginning on line 3 continuing on line 4 as follows: "The corresponding SSR marker for the BNL8.32 locus in bnlg2235." Please amend the sentence to read: "The corresponding SSR marker for the BNL8.32 locus is bnlg1805" in accordance with the correct information elsewhere in the specification on page 52, Table 5, columns 5 and 6, lines 7 under the Chromosome 7 header, and in the 7<sup>th</sup> entry under chromosome 7 in Figure 2. A marked copy and a clean copy of page 29 of the specification are enclosed.

**(2) Claim Objections**

Improper Dependent Form - Claims 44, 53, and 62 are objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Following the suggestion of the Examiner, Claims 44, 53, and 62 are herein amended to independent form.

Wording –Informalities - Claims 45, 54, and 72 are objected to because of wording informalities. The Examiner objects to the use of the term "molecular components" as unclear. In accordance with the Examiner's recommendation, claims 45, 54, and 72 are amended to delete the objectionable wording.

**(2) Claims Rejections – 35 USC § 112**

**Written Description** - Claims 44-61 are rejected under 35 USC § 112 as failing to comply with the written description. Independent claims 44 and 53 are drawn to methods of producing a maize plant wherein said method utilizes the step of backcrossing the trigeneric hybrid at least once to maize. According to the Examiner, the specification only teaches the trigeneric hybrid backcrossed to a *Tripsacum*-teosinte hybrid on page 22 of the specification. The Applicant respectfully points out that steps (a) and (b) of claims 44 and 53 are found on pages 13 and 14 of the specification. The way to produce a plant of step (a) of claim 44 and step (a) of claim 53 is laid out on page 13, lines 16-23 of the specification as follows: "To produce a

maize X *Tripsacum*-teosinte plant or the reciprocal *Tripsacum*-teosinte X maize plant, the intergeneric hybrid plant (*Tripsacum* X teosinte) or (perennial teosinte X *Tripsacum*) is crossed with maize by controlled pollination. In the cross, the pollen of (*Tripsacum* X teosinte) or (teosinte X *Tripsacum*) is transferred to maize silks, or maize pollen is transferred to the silks of (*Tripsacum* X teosinte) or (teosinte X *Tripsacum*).”

The way to make backcrosses to an F<sub>1</sub> trigeneric hybrid of step (a) found in step (b) of claim 44 and step (b) of claim 53 is described on page 14, lines 8-13 of the specification as follows: “To produce a backcross hybrid maize plant, the hybrid plant obtained from maize X (*Tripsacum* X teosinte) or maize X (teosinte X *Tripsacum*) is backcrossed to maize. In the backcross, the pollen of the trigeneric hybrid plant is transferred to the silks of one of the original parents (*Tripsacum* X teosinte) or (teosinte X *Tripsacum*) or maize.”

For further clarification, the Applicant respectfully points out that on page 22, lines 30-35, of the specification, the advanced maize lines (9094 X 7009), (97-5 X 97-1), and V70 are recurrent maize backcross lines. These recurrent maize lines provide literal support for claims 45-52 and 54-61.

**Double Patenting** – Claim 23 is rejected on the ground of nonstatutory obviousness-type double patenting over claim 1 of U.S. Patent No. 6,617,492 B1 (Sept. 9, 2003). A terminal disclaimer in compliance with 37 CFR 1.321 has been filed to overcome this double patenting rejection.

#### **(4) Claims Rejections – 35 USC § 103(a) Obviousness**

(1) Claims 44-70 are rejected under 35 USC § 103(a) as being unpatentable over Eubanks (U.S. Patent No. 5,330,547, July 19, 1994), in view of Eubanks (Theor. Appl. Genet. 94:707-712, 1997). As pointed out by the Examiner, claim 6 of the 1994 patent claims a method of producing a maize plant by cross pollinating maize with either a (*Tripsacum* X teosinte) or (teosinte X *Tripsacum*) recombinant to produce a hybrid plant. However, it is respectfully pointed out that the Examiner’s statement that the 1994 patent claims “backcrossing said hybrid plant at least once to a maize plant” (page 6, line 1, Final Office Action dated 28 April 2008) is incorrect. There is no mention of backcrossing any *Tripsacum*-teosinte-maize hybrid to maize or to a *Tripsacum*-teosinte recombinant in Eubanks’ 1994 patent. No such crosses had been thought of or attempted by Eubanks in 1994. Furthermore, as the Examiner points out, because

“introgression of *Tripsacum* genetic material into maize...has required years of complicated, high risk breeding programs that involve many generations to stabilize desirable *Tripsacum* genes in maize” one of ordinary skill in the art would not have thought that *Tripsacum* genes would be stably inherited in successive backcross generations of maize X *Tripsacum*-teosinte. In point of fact, in 1994, people skilled in the art thought the *Tripsacum* genes would be lost in successive generations.

The Examiner referenced Eubanks (1997) to point out that Doebley et al. (1990) identified over 50 RFLPs associated with quantitative trait loci (QTLs) in maize. Eubanks (1997) employed 15 RFLP markers to genotype two *Tripsacum*-*diploperennis* hybrids designated Sun Dance and Tripsacorn. In accordance with basic genetic principles that a progeny of two parents will inherit an allele from each parent at every genetic locus, Eubanks (1997) examined parental allelic inheritance and reported the number of alleles revealed by the 15 RFLPs in *Tripsacum*, *Z. diploperennis*, the recombinant progeny from crosses between *Tripsacum* and *Z. diploperennis*, and how many of those parental alleles were shared in the progeny. The findings of Eubanks (1997) that the *Tripsacum*-teosinte recombinant plants had inherited alleles from each parent supported the basic principles of inheritance of parental alleles that one skilled in the art would expect. There was no indication in the dataset generated by the 15 RFLPs employed in Eubanks (1997) that precise rearrangements between the parental genomes were creating large numbers of novel alleles. This unprecedented, surprising mutability phenomenon was only subsequently discovered when many more molecular markers were used to screen more *Tripsacum*-teosinte recombinants and *Tripsacum*-teosinte-maize hybrids. Eubanks (1997) underscores that it would not have been obvious to one skilled in the art that: (1) large numbers of novel alleles are generated by crossing *Tripsacum* and teosinte; (2) many of the same novel alleles would be found in progeny of different parents, and (3) the novel chimeric alleles would be stably inherited in subsequent generations of crosses and backcrosses to maize. Neither publication, Eubanks (1994) nor Eubanks (1997), anticipated the unprecedented, novel materials claimed in this application.

(2) Claims 44-70 are rejected under 35 USC § 103(a) as being unpatentable over Eubanks (U.S. Patent No. PP7,977, September 15, 1992), in view of Eubanks (Theor. Appl. Genet. 94:707-712, 1997). Referring to Eubanks 1992, the Examiner wrote: “The claims read on a

method for producing a maize plant, wherein said maize plant is produced by cross pollinating a maize plant with either a (*Tripsacum* X teosinte) or (teosinte X *Tripsacum*) plant to produce a trigeneric hybrid plant, backcrossing said hybrid plant at least once to a maize plant” (page 7, line 19-22, continued on line 1 of page 8, Final Office Action dated 28 April 2008). It is respectfully pointed out, however, that the Eubanks’ 1992 patent does not teach “crossing a maize plant with a (teosinte X *Tripsacum*) plant as the Examiner states (page 8, lines 3-4, Final Office Action dated 28 April 2008). It actually claims a cross between a *Tripsacum* female and a *Z. diploperennis* pollen donor that is an asexually reproducible plant named Tripsacorn. It reports observation of traits in an F<sub>1</sub> hybrid between maize and Tripsacorn, but it does not describe subsequent advanced backcrossed generations. The Examiner cites column 2, lines 9-11 that read “in preliminary field trials of backcrosses to a commercial corn line, drought tolerance and enhanced pest resistance were observed in the F<sub>1</sub> generation.” The use of the word “backcross” is in error because an F<sub>1</sub> hybrid by definition is not a backcross.

The Examiner referenced Eubanks (1997) to point out that Doebley et al. (1990) identified over 50 RFLPs associated with quantitative trait loci (QTLs) in maize. Eubanks (1997) employed 15 RFLP markers to genotype two *Tripsacum*-*diploperennis* hybrids designated Sun Dance and Tripsacorn. In accordance with basic genetic principles that a progeny of two parents will inherit an allele from each parent at every genetic locus, Eubanks (1997) examined parental allelic inheritance and reported the number of alleles revealed by the 15 RFLPs in *Tripsacum*, *Z. diploperennis*, the recombinant progeny from crosses between *Tripsacum* and *Z. diploperennis*, and how many of those parental alleles were shared in the progeny. The findings of Eubanks (1997) that the *Tripsacum*-teosinte recombinant plants had inherited alleles from each parent supported the basic principles of inheritance of parental alleles that one skilled in the art would expect. There was no indication in the dataset generated by the 15 RFLPs employed in Eubanks (1997) that precise rearrangements between the parental genomes were creating large numbers of novel alleles. This unprecedented, surprising mutability phenomenon was only subsequently discovered when many more molecular markers were used to screen more *Tripsacum*-teosinte recombinants and *Tripsacum*-teosinte-maize hybrids. Eubanks (1997) underscores that it would not have been obvious to one skilled in the art that: (1) large numbers of novel alleles are generated by crossing *Tripsacum* and teosinte; (2) many of the same novel alleles would be found in progeny of different parents, and (3) the novel chimeric alleles would be stably

inherited in subsequent generations of crosses and backcrosses to maize. Neither publication, Eubanks (1992) nor Eubanks (1997), anticipated the unprecedented, novel materials claimed in this application.

(3) Claims 71-79 are rejected under 35 USC § 103(a) as being unpatentable over Eubanks (Theor. Appl. Genet. 94:707-712, 1997), in view of Eubanks (U.S. Patent No. PP7,977, September 15, 1992). In consideration of the Examiner's arguments, the Applicant requests claims 71-79 be canceled.

### Concluding Comments

The specification and claims are drawn to a large set of unprecedented, stably inherited novel alleles formed in intergeneric hybrids between *Tripsacum* and teosinte identified by their unique molecular weights as detected by specific RFLP probe-restriction enzyme combinations when compared to the molecular weights of the corresponding parental alleles. Rather than inheriting an allele from each parent in accordance with principles of genetic inheritance in sexually reproducing organisms, the progeny of crosses between *Tripsacum* and teosinte exhibit novel alleles formed at precise genetic loci designated in the application via recombination of their distinctive genomes. These novel or mutant alleles are visualized as bands on autoradiographs and are precisely described by their molecular weight and specific probe-enzyme combination. The genetic material disclosed herein is unprecedented in the plant literature and would **not** have been obvious to one skilled in the art of genetics and plant breeding. As stated in the specification, page 20, paragraph 1, "Such mutant or novel rearrangements in the genetic material are revealed by comparative analysis of the RFLP bands of the parent plants and hybrid progeny. Bands present in the offspring not found in either parent indicate regions of the genome where novel genetic material has arisen, i.e. mutations have occurred...In addition to the rarity and usual deleterious effect of mutations, a basic biological tenet is that mutations occur at random or by chance (Lewin 1997). In a study of spontaneous mutation rates to new length alleles at tandemly repeated loci in human DNA (Jeffreys et al. 1988) mutations arose sporadically and there was no clustering of mutations within a family. Siblings never shared a common mutant allele." Therefore, it is completely unexpected that the same novel alleles recur and are not derived at random or by chance, and that the same novel

alleles are found in hybrid progeny derived from crossing different *Tripsacum* and different teosinte parent plants.”

As stated in the specification, page 23, last paragraph, beginning on line 6, continuing on page 24: “The unique *Tripsacum* polymorphisms and recombinant chimeric RFLPs, their heritability in succeeding generations of *Tripsacum*-teosinte hybrids, and their transmissibility to maize is unprecedented and unexpected based on prior art. Eubanks’ 1992 and 1994 patents make no reference to molecular markers, and Eubanks (1997) is silent as to the unique alleles described in this invention. Because these novel alleles defy everything we know about mutations, mutation frequencies, and heritability in siblings (see detailed discussion above and in the specification, pages 2-3 and page 20), it was **not** obvious to, nor anticipated by one skilled in the art that a high frequency of novel recombinant alleles (i.e. mutations not found in either parent) would be found in progeny derived from crossing *Tripsacum* and teosinte. Nor was it obvious, nor anticipated by, one skilled in the art that the same novel recombinant alleles (i.e. mutations not found in either parent) would be found in progeny from crossing different *Tripsacum* and teosinte plants from different populations and geographical regions. This is unprecedented in the literature, defies the conventional paradigm for allelic inheritance in genetics, and clearly establishes that the prior art would neither anticipate nor render obvious the claimed invention.

Construction of the maize genetic map using RFLPs was possible because each maize parent contributes the same parental allele per locus to the hybrid progeny produced by crossing two inbred lines (Gardiner et al. 1993). In maize, RFLP markers (i.e. probes) detect three or four alleles (i.e. polymorphisms) at each genetic locus in an easily interpretable pattern across a variety of inbred lines (Gardiner et al. 1993, p. 925). In this invention **rather than inheriting an allele from each parent, the progeny of crosses between *Tripsacum* and teosinte exhibit novel alleles formed by intergeneric recombination between the distinctive genomes of *Tripsacum* and teosinte.**

Two of the many examples in the specification illustrate how the materials that are the subject of this application are distinct and unexpected based on the prior art. Referring to the parental alleles for RFLP marker UMC 107-ERI on chromosome 1 of maize, presented in Table 4, page 49 of the specification, when digested with the restriction enzyme *EcoRI*, *Tripsacum* has a 7.9 kb restriction fragment and a 1.5 kb restriction fragment, and *Zea diploperennis* has a 7.1

kb fragment. Based on the genetic principles of inheritance we would expect the *Tripsacum*-teosinte hybrid progeny of those two parents to contain either a 7.9 kb or a 1.5 kb fragment from the *Tripsacum* parent and a 7.1 kb fragment from the teosinte parent. Referring to Table 2, page 35 of the specification, it can be seen that the *Tripsacum* X teosinte recombinant progeny referred to as Tripsacorn contains unexpected novel fragments that are 6.3 kb and 6.1 kb in size. The 6.3 kb novel fragment is also found in the teosinte X *Tripsacum* recombinant progeny referred to as Sun Star. The novel fragments revealed by UMC107-ERI are also found in subsequent crosses between maize and *Tripsacum*-teosinte recombinants designated 64SS, 2019, 3024, and 3125.

A second example of how this invention is unexpected can be seen for RFLP marker UMC 140-ERI on chromosome 1 of maize, presented in Table 4, page 49 of the specification. When digested with the restriction enzyme *EcoRI*, *Tripsacum* has a 10.9 kb restriction fragment and a 7.5 kb restriction fragment, and *Zea diploperennis* has a 2.6 kb fragment. Based on the genetic principles of inheritance we would expect the *Tripsacum*-teosinte hybrid progeny of those two parents to contain either a 10.9 kb or a 7.5 kb fragment inherited from the *Tripsacum* parent and a 2.6 kb fragment from the teosinte parent. Referring to Table 2, page 35 of the specification, it can be seen that the *Tripsacum* X teosinte recombinant progeny referred to as Tripsacorn and teosinte X *Tripsacum* recombinants referred to as Sun Dance Sun Star contain an unexpected novel fragment that is 4.9 kb. The 4.9 kb novel allele revealed by UMC140-ERI is also found in subsequent crosses between maize and *Tripsacum*-teosinte recombinants designated 2019, 3024, 3125, TC64, and 9094 X 7009.

As pointed out in the specification, page 20, paragraph 1, "Such mutant or novel rearrangements in the genetic material are revealed by comparative analysis of the RFLP bands of the parent plants and hybrid progeny. Bands present in the offspring not found in either parent indicate regions of the genome where novel genetic material has arisen, i.e. mutations have occurred...mutations are rare, and in most cases deleterious. Broadly speaking among all organisms, mutation rates vary and they range from 1 in 1,000 to 1 in 1,000,000 gametes per generation depending on the gene involved (Curtis and Barnes 1989). For example, each human with approximately 100,000 genes is expected to carry 2 mutant alleles. The unique restriction fragments of the *Tripsacum*-teosinte hybrids occur at 148 out of 176 loci and are unprecedented in their high mutation rate. Furthermore, the novel polymorphisms are stably inherited in

succeeding generations of *Tripsacum*-teosinte progeny and of maize by *Tripsacum*-teosinte progeny. In addition to the rarity and usual deleterious effect of mutations, a basic biological tenet is that mutations occur at random or by chance (Lewin 1997). In a study of spontaneous mutation rates to new length alleles at tandemly repeated loci in human DNA (Jeffreys et al. 1988) mutations arose sporadically and there was no clustering of mutations within a family. Siblings never shared a common mutant allele. Thus it is unexpected that the same mutations would recur not only among siblings but among hybrids of different parentage.” The demonstration of the same unique polymorphisms repeatedly found in hybrid progeny derived from crossing different *Tripsacum* and different teosinte parent plants runs counter to genetic dogma. This phenomenon is unprecedented in the literature, defies the conventional paradigm for parental allelic inheritance in genetics, and clearly establishes that the prior art would neither anticipate, nor render obvious, the claimed invention.

Claims 44-63 are currently amended. The amended claims, along with the above responses to wording informalities, obviousness-type double patenting, and written description are presented to clarify the distinctive, unprecedented, novel material being claimed in order to overcome the Examiner’s rejections, and to bring the application into proper order for allowance of the claims.

Respectfully submitted,

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